Flash Purification of non-UV-active Compounds by Evaporative Light Scattering Detection

Application Note

Authors

Linda Lloyd, Stephen Ball
and Keeley Mapp
Agilent Technologies, Inc.

Introduction

Flash chromatography is a rapidly growing technique in the field of chromatography, and is essentially a simplified, rapid form of preparative LC. As with traditional HPLC, the analysis of compounds that do not have a UV chromophore can cause problems due to their very low limit of detection. Post-column addition of a UV-active fragment, or fluorescing agent, is one option. However, a much simpler solution to this problem is the use of an evaporative light scattering detector (ELSD) coupled to the Flash instrument.

This note demonstrates the benefits of using a Agilent 385-ELSD with an Agilent 971-FP Flash instrument for the analysis of a sample containing a mixture of UV and non-UV-active compounds. The major advantage of dual detection is the ability to prevent loss of sample components that remain undetected by UV, and are therefore not isolated by the fraction collector but are lost to waste.

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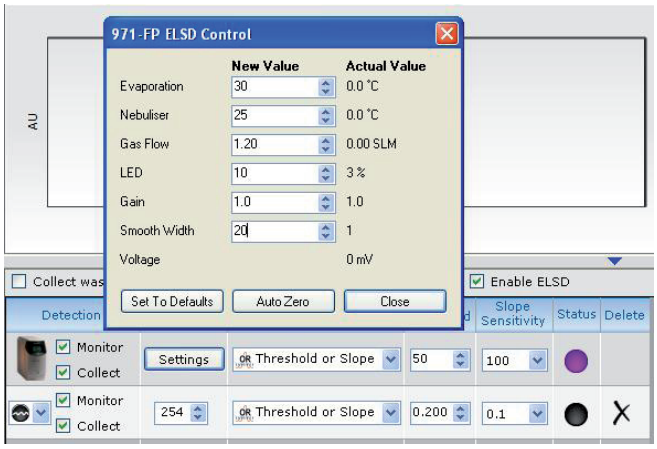


Figure 1. The Agilent 971-FP ELSD control window

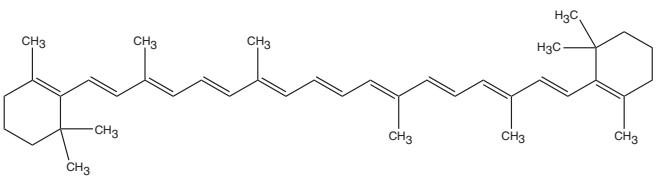
Software Control

The 971-FP uses software to incorporate an ELSD as an alternative method of detection, and these two instruments in combination are powerful tools in the rapid screening and purification of crude samples. The 971-FP software permits direct control of the ELSD via an on-screen user interface (Figure 1), letting the operator change detection settings as and when required. The ELSD response can be used to monitor or trigger fraction collection.

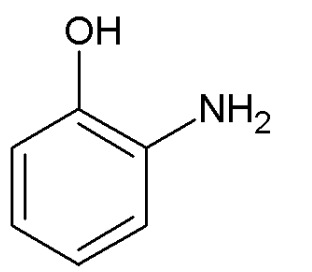
Methods and Materials

A solution containing 10 mg/mL of β-carotene, α-tocopherol, δ-tocopherol, 2-aminophenol and 3-aminophenol in hexane was made up, and injected onto a SuperFlash column.

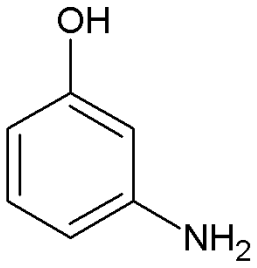
Column:	SF15-12g Si 35 (part number AX1371-7)
Eluent A:	Hexane
Eluent B:	Ethyl acetate
Gradient:	0 - 100%B in 10 min
Flow Rate:	18 mL/min
Inj Vol:	0.5mL
Temp:	Ambient
Detection:	UV at 254 nm, ELSD (neb=15C, evap=30 °C, gas=1.2 SLM), LED=3%



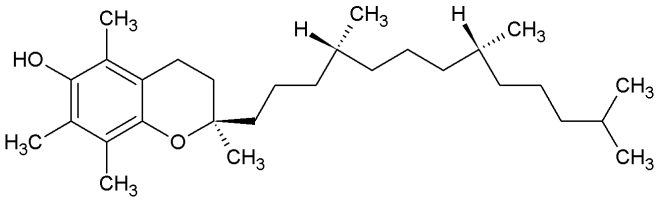
β-carotene



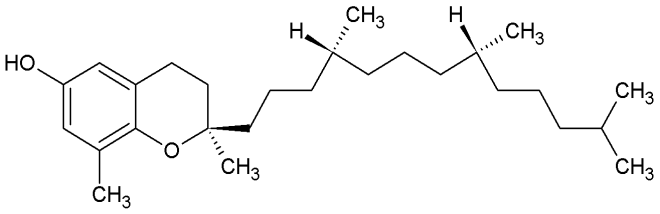
2-aminophenol



3-aminophenol



α-tocopherol



δ-tocopherol

Results and Discussion

Figure 2 is an overlay of the different detector responses. The chromatogram was exported directly from the Agilent 971-FP, and highlighted the importance of using an additional detection technique. The UV response showed only two, very small peaks, for 2-aminophenol and 3-aminophenol, and so if only this output was used to trigger fraction collection, some of the components in the sample would be lost to waste.

The ELS detector picked up additional peaks at 1 and 4 minutes, which corresponded to β-carotene, α-tocopherol and δ-tocopherol, respectively. Fraction collection was also triggered from the both the UV and ELSD responses. Therefore, all components were now collected. The sensitivity was also significantly increased, and the baseline was much less noisy, so giving improvements in the signal to noise ratio and improved visibility for trace components.

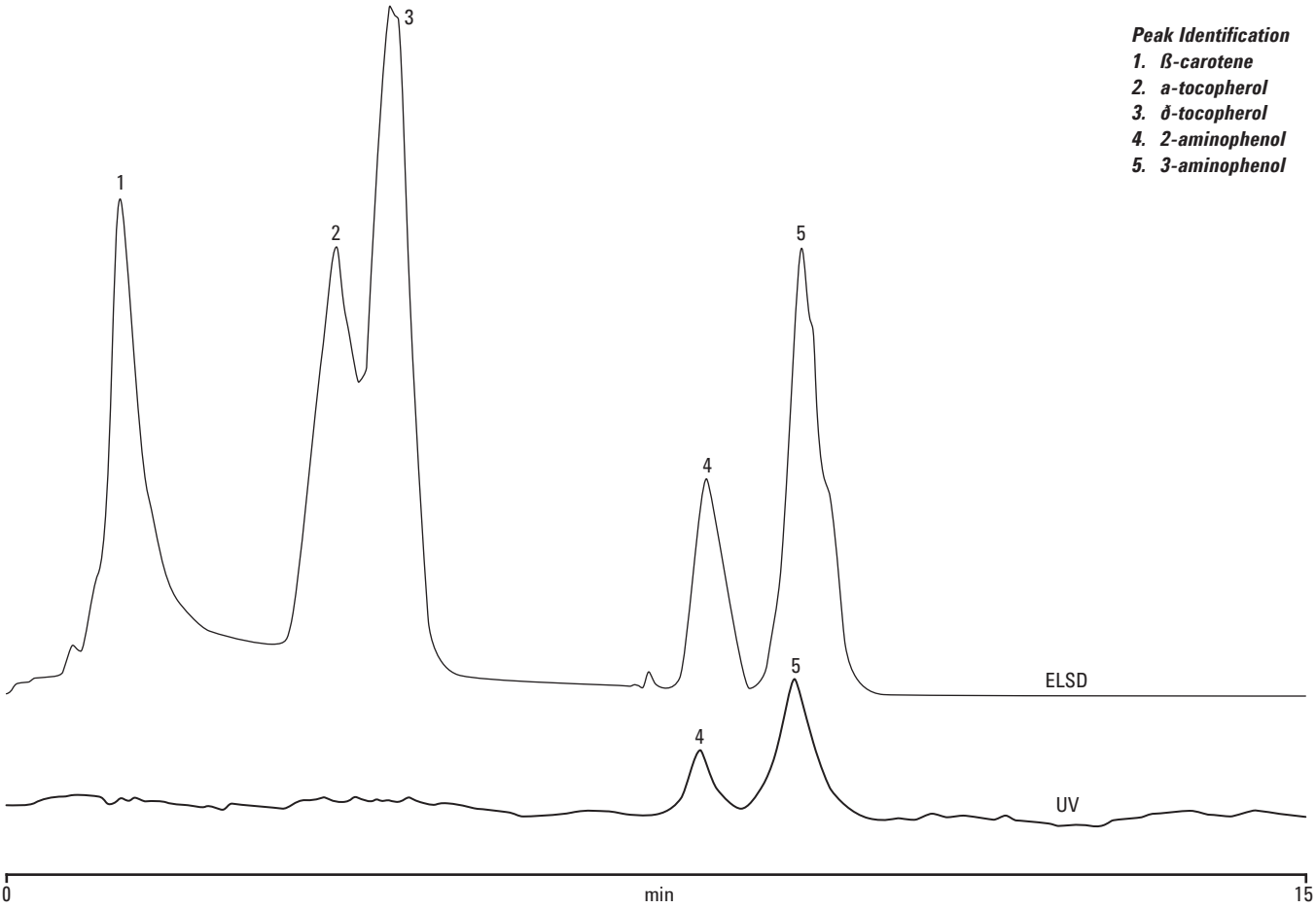


Figure 2. Analysis of UV and non-UV active compounds using a SuperFlash SF15 - 12 g Si 35 column

Conclusion

When using Flash purification for compounds with little or no chromophore, the combination of ELSD and UV detectors is a powerful technique that maximizes purification efficiency. The Agilent 971-FP monitors both UV and ELSD responses simultaneously, ensuring that valuable sample is not lost due to poor UV visibility of individual compounds as fraction collection is triggered by both signals.

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